Klinik für Kleintiermedizin
der Vetsuisse-Fakultät der Universität Zürich
Direktorin: Prof. Dr. Claudia Reusch

# *SLC3A1* and *SLC7A9* Mutations in Autosomal Recessive or Dominant Canine Cystinuria: A New Classification System

**Inaugural-Dissertation**

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

**Ann-Kathrin Brons**

Tierärztin
von Flensburg, Deutschland

genehmigt auf Antrag von
Prof. Dr. Urs Giger, Referent

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## Summary

Cystinuria, one of the first recognized inborn errors of metabolism, has been reported in many dog breeds. The objective was to determine urinary cystine concentrations, inheritance and mutations in the *SLC3A1* and *SLC7A9* genes associated with cystinuria in 3 breeds: Mixed and purebred Labrador Retrievers (n=6), Australian Cattle Dogs (6), Miniature Pinschers (4) and one mixed breed dog with cystine urolithiasis, relatives and control dogs. Urinary cystinuria and aminoaciduria was assessed and exons of the *SLC3A1* and *SLC7A9* genes were sequenced from gDNA. In each breed male and female dogs, independent of neuter status, were found to form calculi. A frameshift mutation in *SLC3A1* resulting in a premature stop codon was identified in autosomal-recessive (AR) cystinuria in Labrador Retrievers and mixed breed dogs. A 6 bp deletion removing 2 threonines in *SLC3A1* was found in an autosomal-dominant (AD) cystinuria with a more severe phenotype in homozygous than heterozygous Australian Cattle Dogs. A missense mutation in *SLC7A9* was discovered in an AD cystinuria in Miniature Pinschers with only heterozygous affected dogs observed to date. Breed specific DNA tests were developed. These studies describe the first AD inheritance and the first putative *SLC7A9* mutation to cause cystinuria in dogs and expand our understanding of this phenotypically and genetically heterogeneous disease, leading to a new classification system for canine cystinuria and better therapeutic management and genetic control in these breed.



Keywords: metabolic disease, uroliths, nephropathy, hereditary disease

# Zusammenfassung

Die Cystinurie, eine der zuerst beschriebenen erblichen Stoffwechselerkrankungen, wurde bei vielen Hunderassen dokumentiert. Ziel war die Bestimmung der Cystinausscheidung im Urin, Erbgänge und Mutationen in den Genen *SLC3A1* und *SLC7A9* im Zusammenhang mit einer Cystinurie bei 3 Rassen: Labrador Retriever und –Mischlinge (n=6), Australian Cattle Dogs (6), Zwergpinscher (4) und ein Mischling mit Cystinsteinen, sowie verwandte Tiere und Kontrollen. Dazu wurde die Ausscheidung von Cystin und anderen Aminosäuren mit dem Harn bestimmt und die Exons der Gene SLC3A1 und SLC7A9 aus gDNA sequenziert. Hunde jeder Rasse bildeten unabhängig von Geschlecht und Kastrationsstatus Cystinsteine. Eine Nonsense-Mutation in *SLC3A1,* resultierend in einem vorzeitigen Stop-Codon, wurde bei einer autosomal-rezessiven Cystinurie bei Labrador Retrievern und –Mischlingen gefunden. Eine Deletation von 6 bp mit dem Fehlen von zwei Threonin Molekülen in *SLC3A1*, wurde bei einer autosomal-dominanten (AD) Cystinurie mit stärker ausgeprägtem Phänotyp bei homozygoten als heterozygoten Australian Cattle Dogs festgestellt. Eine Missense-Mutation in *SLC7A9* bei einer AD Cystinurie mit bisher nur heterozygot erkrankten Tieren, wurde beim Zwergpinscher beobachtet. Rassespezifische DNA-Tests wurden entwickelt. Diese Studie beschreibt erstmals AD Erbgänge der Cystinurie des Hundes, sowie eine ursächliche Mutation im Gen *SLC7A9*. Sie erweitert das Verständnis dieser phäno-und genotypisch variablen Erkrankung und resultiert in einer neuen Klassifikation der caninen Cystinurie.

Schlüsselwörter: Metabolische Erkrankung, Harnsteine, Nephropathie, Erbkrankheit

# Abbreviations

| | |
|---|---|
| AD | autosomal dominant |
| AR | autosomal recessive |
| bp | base pair(s) |
| DNA | deoxyribonucleic acid |
| EDTA | ethylenediaminetetraacetic acid |
| COLA | cystine, ornithine, lysine and arginine |
| PCR | polymerase chain reaction |
| SNP | single nucleotide polymorphism |
| UV | ultra violet |

# Introduction

Cystinuria (OMIA 000256-9615) is one of the first inborn errors of metabolism recognized by Sir Archibald Garrod [1,2] and is an inherited selective renal transport defect [3,4] involving cystine and the dibasic amino acids ornithine, lysine, and arginine, collectively known as COLA.[4] In contrast to the normal near complete reabsorption of COLA in the proximal renal tubules, these amino acids reach high concentrations in the urine, where only cystine causes a clinical problem.[5] The low solubility of cystine in acidic and neutral urine may lead to the formation of cystine crystals and uroliths in the urinary tract,[6] which result clinically in stranguria, hematuria, urinary obstruction and renal failure.[7,8]

In 1823, cystinuria was the first reported "inborn error of metabolism" in dogs,[9] and now is known to affect >70 canine breeds according to reports of veterinary urolith analysis laboratories worldwide.[10,11] Based upon the varied clinical presentations, metabolic derangements and genetic studies, we previously divided canine cystinuria into type I and non-type I cystinuria:[12] type I cystinuria, as characterized in Newfoundlands and Landseers,[13] causes massive aminoaciduria of COLA and juvenile to adult calculi formation. It is an autosomal recessive trait affecting both males and females independent of neutering status, although more often cystinuric males show clinical signs of urinary obstruction, presumably due to anatomical differences between males and females.[13] In non-type I cystinuria, exemplified by Mastiffs and related breeds, Scottish Deerhounds and Irish Terriers, only intact adult males show variable degrees of aminoaciduria and the average age of stone formation is later than in Newfoundlands. The molecular basis and mode of inheritance of non-type I cystinuria remain unknown, but is not an X-chromosomal disorder and appears to be testosterone dependent. [a,14]

Two genes, *SLC3A1* and *SLC7A9* encode the polypeptide subunits of $b^{0,+}$, the basic amino acid transporter system (for reviews see [4,15,16]). The *SLC3A1* gene encodes the extracellular heavy chain referred to as rBAT, and the *SLC7A9* gene the light chain called $b^{0,+}AT$.[3] The subunit $b^{0,+}AT$ has 12 transmembrane domains typical of cell membrane transporters and heterodimerizes with rBAT exclusively to form the COLA amino acid transporter.[17] Among all the cystinuric dogs, to date only one mutation in *SLC3A1,* an early stop codon precluding the production of the rBAT protein, therefore leading to the loss of $b^{0,+}$ function, was identified in cystinuric Newfoundlands in 2000.[18] And while the disease was widely recognized in the breed, screening programs for the mutation have drastically reduced the incidence of cystinuria in Newfoundlands and Landseers worldwide.

We report here the clinical, metabolic and molecular genetic characterization of cystinuric Labrador Retrievers, Australian Cattle Dogs, Miniature Pinschers and mixed breed dogs. We identified two new *SLC3A1* mutations*,* one inherited in an autosomal dominant fashion and the other showing autosomal recessive inheritance, and the first *SLC7A9* mutation found in dominantly inherited cystinuria in dogs, thereby characterizing the genetic heterogeneity of cystinuria in dogs at the gene mutation level. Because our data now demonstrate multiple genetic etiologies and modes of inheritance for canine cystinuria, we propose a new expanded classification system for canine cystinuria.

# Materials and Methods

## Animals and Samples

Dogs of 3 different breeds and mixed breed dogs presented with cystine uroliths and relatives of these dogs (Labrador Retrievers n=11, Australian Cattle Dogs n=10, Miniature Pinschers n=13, mixed breed dogs n=3) were included in the study. Free catch urine and EDTA-anticoagulated blood samples were sent chilled or frozen to the Metabolic Genetics Laboratory at the University of Pennsylvania.[b] Archival urine and EDTA-blood samples were used as controls. Medical records and pedigree information were reviewed, as far as provided. These studies were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

## Urinary Metabolic Tests

Urine samples were chilled or frozen until analysis for cystine using the semi-quantitatively cyanide-nitroprusside method and/or quantitatively by complete amino acid analysis using an automated amino acid analyzer[c] with ninhydrin detection validated for canine urine samples. Reference ranges were created using free-catch urine samples obtained from adult dogs of breeds known not to be renal stone formers. The urinary nitroprusside test is a simple screening test detecting the presence of saturing concentrations of >312 µmol cystine / l urine, based upon a chemical reduction reaction of cystine to cysteine by cyanide. A positive reaction is visible as a color change of the urine from yellow to magenta red.[19]

## Molecular Genetic Studies

Genomic DNA was extracted from EDTA-blood samples using the Midi kit[d] or phenol/chloroform extraction. Primer pairs to amplify exons and surrounding splicing

consensus sequences of the *SLC3A1* and *SLC7A9* genes were designed using the UCSC-genome primer browser[e] or the LaserGene DNA sequence analysis package[f] and optimal annealing and amplification conditions were established (Table 1).

*SLC3A1* exons 2-10 were amplified in a polymerase chain reaction (PCR) of a total volume of 50 µl containing 35.5 µl of DNAse-free water, 5 µl of 10X Ex Taq Buffer, 5 µl of dNTPs (2.5 mM each), 1 µl each of the 40 µM forward-and reverse primers, 0.5 µl of an Takara Ex Taq HS Polymerase (5 U/µl)[g] and 2 µl of the gDNA template. For the amplification of *SLC3A1* exon 9, 12.5 µl of an EmeraldAmp Max HS PCR Master Mix (2X)[g] was set up with 9.5 µl of DNAse-free water, 1 µl each of the forward and reverse primer (100 mM) and 1 µl of the gDNA template.

For *SLC7A9* exons 9-11 and 13, the same PCR reaction containing the Takara Ex Taq HS Polymerase[g] was used. For the DNA fragments containing exons 2, 7-8 and 12, a 30 µl PCR mix containing 1X Buffer B, with 10 µl of DNAse-free water, 5 µl 5X Buffer B, 5 µl dNTPs , 1 µl each of the 20 µM forward- and reverse primers, 1 µl AkkuPrime[h] GC- Rich DNA Polymerase (2 U/µl) and 2 µl of the gDNA template. For *SLC3A1* exon 1 as well as *SLC7A9* exons 1 and 3-6, a different PCR mix of 25 µl total volume containing 5 µl of DNAse-free water, 12.5 µl 2X Xtreme buffer, 5 µl dNTPs (2 mM each), 0.75 µl each of the 20 µM forward-and reverse primers (for SLC3A1 exon 1 0.375 µl of a 100mM primer pair), 0.5 µl Novagen KOD Xtreme Hot Start DNA Polymerase (1 U/µl)[i] and 0.5 µl of the gDNA template was used.

All amplifications with the Ex Taq Hot Start Polymerase[g] and the GC-rich polymerase[h] were performed with a PCR Mastercycler proS[j] and the following sequential steps: initial denaturation at 98°C for 3 minutes, followed by 35 cycles of amplification with denaturation at 98°C for 45 seconds, annealing of primers at corresponding temperature for 30 seconds and extension at 72°C for 30 seconds with a final extension at 72°C for 7 minutes.

For the PCR-reaction with Novagen KOD Xtreme Hot Start DNA Polymerase,[i] the amplification was performed using an GeneAmp PCR system 2700[k] with the following sequential steps: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of amplification with denaturation at 98°C for 1 second, annealing of primers at 60°C for 10 seconds and extension at 68°C for 1 minutes and 37 seconds, except for the amplification of *SLC3A1* exon 9. Here, a touchdown PCR with an initial denaturation at 95°C for 3 minutes, 10 cycles of denaturation at 95°C for 30 seconds, and a touchdown annealing for 1 minute starting at a temperature of 65°C and lowering by 1°C after each cycle and an extension for 1 minute at 72°C, followed by another 25 cycles with an annealing at 55°C for 30 seconds, was performed on the same instrument.

Following amplification, each PCR product together with a loading dye was electrophoresed on a 2% agarose gel containing ethidium bromide. Under UV light exposure, observed DNA bands were excised and purified using a QIAquick Gel Extraction kit[d], and submitted for sequencing at the University of Pennsylvania's Core DNA Sequencing Facility on an Applied Biosystems[k] instrument. The sequences were compared to the CanFam 3.1 canine reference genome assembly using the NCBI-Blast-tool[l] and chromatograms were displayed for visual examination using the ChromasPro[m] sequence viewer. All exonic and intronic flanking as well as the 5' and 3' untranslated regions of both genes were analyzed.

Genetic nomenclature is in accordance with the Guidelines and Recommendations for Mutation Nomenclature by the Human Genome Variation Society.[20]

**DNA Mutation Tests**

TaqMan SNP genotyping assays[j] were developed for the mutations found in Labrador Retrievers and Miniature Pinschers. For the deletion found in Australian Cattle Dogs, a PCR assay was developed, using the specific primer pair (5'GTTTCCAAAGGACACGGTCAC'3

and 5’GTTACCTGTATCTCCCAGGCTC’3), that discriminates the normal and affected alleles by electrophoresis on a 5% polyacrylamide gel.

## Breed and Mixed Breed Determination

Most dogs were considered as purebred as reported by owners and with registration and pedigree information provided in some cases. Breed or composition of specific mixed breed dogs was assessed by the Wisdom Panel Test.[n]

# Results

## Clinicopathological Findings

Dogs with cystine urolithiasis included 4 purebred (LABR 1-4) and 2 mixed breed (LABX 1-2) Labrador Retrievers, 6 Australian Cattle Dogs (AUCD 1-6), one mixed breed dog (MIXB 1), and 4 Miniature Pinschers (MINP 1-4). Control dogs with no clinical signs of cystinuria included Labrador Retrievers (LABR 5-7 related to LABR 4 and LABR 8-11), Australian Cattle Dogs (AUCD 7-10 related to AUCD 2) and Miniature Pinschers (MINP 5-13, all related to MINP 1-4) and historical urine amino acid analyses. Uroliths, surgically removed by cystotomy, consisted of 100% cystine, based on crystallographic analyses by various commercial urolith laboratories.

All 5 male mixed and purebred Labrador Retrievers, 4 of them castrated by at least 3 months prior to testing, developed voiding problems due to cystine calculi at ages ranging from 6 to 14 months. The spayed female LABR 4 showed no evidence of cystine calculi in bladder- and kidney until 7 years of age, requiring unilateral nephrectomy and cystotomy at that time (Table 2). Available dogs related to LABR 4 did not show any urinary tract signs and were 7, 12 and 13 years old at the time of testing. Dogs in an additional Labrador family (LABR 8-11), related to a cystine stone former that was not available for the study, also showed no urinary tract signs.

Among the Australian Cattle Dogs studied, all from different parts of the United States, 2 neutered males and one intact male developed urinary tract signs due to cystine uroliths within their first year of life, whereas the 2 other affected males and the female did not show urinary obstruction until 4-5 years of age (Table 2). The available dogs related to AUCD 2 did not show any clinical signs of cystinuria.

In addition, a male castrated mixed-breed dog (MIXB 1), obtained from a shelter, experienced urinary obstruction due to cystine calculi twice by 2 years of age. This dog has been managed with urinary alkalization, diet, and tiopronin[o] and has since not obstructed again as of the time of this study, at age 5 years.

The family of 13 Miniature Pinschers (MINP 1-13) was comprised of 7 male dogs, one of them neutered, and 6 intact females. Two males, one intact and one neutered, as well as 2 females had been diagnosed with problems urinating due to cystine uroliths and crystalluria. MINP 1 first developed signs of cystinuria at 11 months of age, whereas the other male and the females were first recognized as affected at the age of 2 years and older (Table 2). All other related dogs showed no clinical signs of cystinuria.

**Metabolic Urine Analyses**

Urine samples from Labrador Retrievers and Australian Cattle Dogs with a history of cystine calculi formation were all strongly positive by the cyanide-nitroprusside test, whereas this test was negative for the related dogs of either breed and also all unrelated dogs, if tested. These stone formers included male and females dogs and the cystine test results were positive even after neutering the dogs. For the Miniature Pinschers urine samples were not available for the cyanide-nitroprusside test.

Similarly quantitative amino acid determination revealed increased urinary cystine, ornithine, lysine and arginine concentrations and thus high COLA values (Table 2), but normal urinary concentrations for other amino acids (not shown). In one stone-forming dog (LABR 4) cystine was found by the nitroprusside test, but gave a normal quantitative cystine result, perhaps due to precipitation of excess cystine in the urine during storage or laboratory error. Interestingly, among the early Australian Cattle Dog stone formers the COLA values were at least twice as high as those from the male later stone formers. Urinary nitroprusside

test results were negative (COLA values were not determined) for all related Labrador Retrievers and Australian Cattle Dogs that showed no clinical signs of cystinuria. In the Miniature Pinschers, all 4 cystine stone formers as well as 1 other male were strongly cystinuric, and 1 male and 2 females had moderate elevations of COLA concentrations. The degree of elevation of urinary COLA concentrations did not correlate with the observation of stone formation or the age. Of note, some cystinuric Miniature Pinschers showed higher urine cystine concentrations than the combined concentrations for ornithine, lysine, and arginine.

## DNA Sequencing and Mutation Analyses

All exonic DNA sequence differences in *SLC3A1* and *SLC7A9* gene between the CanFam 3.1 canine reference genome assembly and cystine stone-forming dogs LABR 2, LABX 1, AUCD 1 and 5, MIXB 1 as well as MINP 8 are shown in Table 3. Neither the mutation found to cause cystinuria in the Newfoundlands,[18] nor the amino acid exchanges formerly described polymorphisms in exon 6 (I192V) and 10 (S698G) of *SLC3A1* and exon 5 (A217T) of *SLC7A9* in English Bulldogs were observed in any of the dogs studied here.[21] The sequence differences resulting in cystinuria are below detailed for each breed studied.

**Mixed and Purebred Labrador Retrievers** - A homozygous 1 bp deletion (c.350delG) in exon 1 of the *SLC3A1* gene was found in the DNA from the one cystinuric Labrador Retriever and one Labrador mixed breed dog (LABR 2 and LABX 1). The deleted guanine in codon GGC (coding for glycine) causes a shift of the open reading frame (p.Gly117Alafs*41) leading to a premature stop codon 41 codons later (Fig. 1A).

Based on *SLC3A1* exon 1 sequencing, the additional 3 cystinuric Labrador Retrievers as well as the cystinuric Labrador-mixed breed dog were also homozygous for the single base deletion. Among the related non-cystinuric Labrador Retrievers tested, the parents of the affected LABR 4 were heterozygous for the deletion, while the 1 available littermate had 2

normal alleles. In the unrelated Labrador family (LABR 5-8) 1 parent and 1 daughter were carriers for the mutation. Exon 1 of the canine *SLC3A1* contains 2 polymorphic hexamer repeat sequences, GAGCCC and GTCGGG, which have been shown to be polymorphic within and between dog breeds.[18,21] All sequenced Labrador Retrievers and Labrador Retriever mixed breed dogs were homozygous and had 8 copies of the GAGCCC sequence and 6 copies of the GTCGGG sequence (referred to as haplotype 8/6).

**Australian Cattle Dogs –** A heterozygous deletion of 6 bases (c.1095_1100del) was found in exon 6 of the *SLC3A1* gene from AUCD 2. This deletion removes 2 of 3 consecutive threonine residues (p.Thr366_Thr367del) from the rBAT polypeptide encoded by this gene (Fig. 1B). Sequencing of this exon in other cystinuric Australian Cattle Dogs showed that 4 were homozygous (AUCD 1, 3-4 and 6) and 2 were heterozygous (AUCD 2 and AUCD 5) for this deletion. Two dogs related to AUCD 2 were homozygous for the normal allele, whereas 1 young son was also heterozygous for the deletion (lost to follow up and no urine available for study). Only the 7/6 haplotype for the hexamer repeats in exon 1 was observed in the sequenced Australian Cattle Dogs.

**Mixed Breed Dog -** Interestingly, the cystinuric dog MIXB 1 had the same homozygous 6 bp deletion in exon 6 of the *SLC3A1* gene as found in the Australian Cattle Dogs. This was an unexpected finding, given the physical appearance of MIXB 1. Because of the simple sequence repeat nature of the 6 bp deletion, potentially predisposing this region for a high mutation rate, we examined the possibility that the same deletion event occurred independently in Australian Cattle Dogs and in the ancestors of MIXB 1. Examination of all exons and some flanking intronic regions in the *SLC3A1* gene revealed that this dog was entirely homozygous for the same silent and intronic polymorphisms (Table 3) observed in the homozygous affected Australian Cattle Dogs, compared to the canine reference genome sequence. The hexomeric repeat haplotype was 7/6 as seen in the Australian Cattle Dogs.

**Miniature Pinschers** – In exon 9 of the *SLC7A9* gene, a single base change (c.964G>A*)* was detected, causing the substitution of a large positive charged, hydrophilic arginine for the very small, hydrophobic glycine residues, (p.Gly322Arg) (Fig. 1C). All additional cystinuric Miniature Pinschers (as defined by stone formation or elevated urine COLA levels) studied were found to be heterozygous for this missense mutation, whereas all dogs homozygous for the normal allele had normal urinary COLA levels and no history of calculi formation (Fig. 2). The exonic sequence of the *SLC3A1* gene did not reveal any mutations and the observed number of hexomeric repeats in exon 1 was 7/5 in all Miniature Pinschers tested.

## DNA Mutation Tests

The presence of the mutations in Labrador Retrievers and Miniature Pinschers can be easily detected by the established TaqMan SNP genotyping assays[k] and retesting all studied DNA samples by this method confirmed the genotype results from sequencing.

A simple DNA amplification of a small region surrounding the 6 bp deletion found in Australian Cattle Dogs, readily differentiates between the normal and mutant allele due to the 6 bp fragment size difference and can be used as a screening test to discriminate heterozygous or homozygous affected or normal genotypes (Fig. 3).

## Breed and Mixed Breed Determination

Because these molecular genetic studies included purebred and mixed breed dogs, the breed composition of some dogs was examined using the Wisdom Panel Test.[n] The cystinuric Labrador mixed breed dogs were reported to have Labrador contributions. The 2 heterozygous cystinuric Australian Cattle Dogs were found to be purebred Australian Cattle Dogs, as stated by their owners.

The mixed breed dog (MIXB 1), weighing 18.5 lbs and measuring 16.5 inches at the shoulder with a white soft long hair coat, had a breed constitution of 1/4 each from Miniature Poodle, Chihuahua, and Shi Tzu, with several other breeds constituting the last quarter, but no evidence of Australian Cattle Dog. Most interesting despite the many breeds involved, this dog had a high degree of homozygosity (69%) for the informative 312 single nucleotide polymorphism (SNP) markers, which typically is seen with inbred and purebred dogs according to extended report of the breed testing results.

The Miniature Pinschers were not subjected to breed testing as these dogs were registered with official pedigrees showing common ancestry. Interestingly, these dogs were from Europe (mostly from Germany) and belonged to a single 5 generations pedigree (Fig. 2).

## Discussion

While cystinuria is one of the classic inborn errors of metabolism and has been described in several species,[12] the disease is phenotypically heterogeneous in humans and animals, and its molecular basis is not yet completely understood.[12,22,23] The genetic studies in 3 breeds presented here document some of the heterogeneity in canine cystinuria. We identified a previously undescribed mutation in the *SLC3A1* gene in an autosomal recessive form of the disease, phenotypically and genetically similar to that previously described in Newfoundland dogs.[13,18] We also characterized the first examples of both *SLC3A1* and *SLC7A9* gene mutations that are associated with autosomal dominant patterns of inheritance of cystinuria in dogs. These genes together encode the heteromeric $b^{0,+}$ amino acid transporter.[16,17] Screening for hexagonal crystalluria on microscopic sediment evaluation,[24] crystallographic calculi analysis, urinary nitroprusside test for cystine and/or amino acid quantitation for increased COLA readily detected the cystinuric dogs in this study, and genetic tests can now also be used. Unfortunately, we did not have access to kidneys of any cystinuric dogs for protein and mRNA studies, but computer modeling of the observed mutations and current knowledge of the protein structure and functional domains strongly suggest a defective reabsorption of the transporter system $b^{0,+}$. Based upon the genetic findings, we propose a novel classification nomenclature for cystinuria in dogs (Table 4), which also has important implications for management and genetic control.

Similar to the cystinuria in the Newfoundland (and Landseer) breed, which was the first cystinuria characterized at the molecular level in any dog breed, cystinuria in **Labrador Retrievers** is caused by an early frameshift mutation (c.350delG) in the *SLC3A1* gene, also leading to a premature stop codon that truncates the rBAT protein to 157 amino acids instead of 784 (Fig. 1A). The early termination may also cause accelerated RNA decay and reduced

or no protein synthesis. Regardless of the amount of protein, the truncated protein would only contain 13 amino acids from the extracellular domain, and would likely be unstable and unable to dimerize precluding normal function. Several human type I cystinuric patients, with mutations in the amino terminal region of the protein that cause a premature stop further from the amino terminus than in the Labrador Retrievers (and Newfoundlands), show partial or complete loss of the rBAT protein.[25] Only Labrador Retrievers homozygous for the c.350del mutation were cystinuric and both males and females, regardless of neuter status, were cystinuric and developed cystine calculi early in life, albeit more frequently and earlier in males (by 1.1 years in this study) than females, presumably due to anatomic differences (Table 2). Interestingly, the spayed female Labrador Retriever dog developed nephrolithiasis, which has also been reported in cystinuric Newfoundland dogs,[13] but is generally rarely reported in dogs with any type of uroliths, in contrast to human patients.

Moreover, several related Labrador Retrievers were heterozygous for this nonsense mutation in the *SLC3A1* gene, but neither showed clinical urinary tract signs nor positive nitroprusside urine tests. This is consistent with an autosomal recessive mode of inheritance as previously reported for the Newfoundland breed.[13,18] Based upon information from urolith analysis laboratories and particularly when considering the large popularity of the Labrador Retriever breed, the incidence of cystinuria in this breed appears low.[8,26] In contrast, a breed predisposition to cystinuria had been recognized in the Newfoundland breed, with at least one known popular stud dog that was either cystinuric.[18] While the information presented here can be used to devise genetic tests differentiating between affected, carrier (asymptomatic) and normal dogs, general screening of the population of pedigreed Labrador Retrievers is currently not recommended. Those Labrador Retrievers showing clinical signs of cystinuria and any related dogs should be screened by urine testing followed by DNA testing. And since the mutation seems rare, it is reasonable to consider elimination of carrier and affected dogs

from the large breeding stock of Labrador Retrievers without depleting the gene pool of the breed.

The cystinuric **Australian Cattle Dogs** had an in-frame 6 bp deletion removing 2 of the 3 adjacent threonine residues in exon 6 of the *SLC3A1* gene (Fig. 1B). This mutation may have occurred during DNA replication by mispairing or polymerase "slippage" within the repeat of a CAC sequence. This portion of the rBAT protein is found in a highly conserved portion of the extracellular domain among all mammals and other vertebrates (Fig. 4A). Due to the fact that the deleted amino acids are in the extracellular part of the rBAT protein, which is responsible for substrate trafficking,[27] this deletion may interfere with normal transport function, but is not predicted to prevent heterodimerization between rBAT and $b^{0,+}$AT as the binding and anchoring sites remain unaffected. All dogs, male and female, homozygous or heterozygous for this deletion were cystinuric, but all homozygous males and the one homozygous female had higher urinary cystine and COLA values than the heterozygous dogs (Table 2). Moreover, the homozygous males showed obstruction earlier in life than heterozygous male Australian Cattle Dogs. The later age of onset of clinical signs in the homozygous female Australian Cattle Dog may be explained by the differences in gender-related urinary tract anatomies. The heterozygous offspring of AUCD 2 was 3 months old when tested negative by nitroprusside test and he was unfortunately not available for follow up cystine and COLA testing. As the tested mother showed a normal genotype and cyanide nitroprusside test, the father of AUCD 2 was likely heterozygous for the deletion and therefore cystinuric, but he was also not available for study. We sequenced all exons of the *SLC3A1* and *SLC7A9* genes from homozygous and heterozygous Australian Cattle Dogs and did not discover any additional variations in the coding sequence in either allele or gene (Table 3). This excludes the possibility of having a compound heterozygote with 2 different mutations in the same gene (common in people) or one in the *SLC3A1* and one in the *SLC7A9*

gene.[28] From that we infer that cystinuria in the Australian Cattle Dog is inherited in an autosomal dominant trait. No 6 bp deletion in the same region of the *SLC3A1* gene has been reported in human patients (the human sequence does not contain the triplet repeat), but a large duplication of 5 exons involving the A domain has been shown to cause severe cystinuria in homozygous and heterozygous patients.[25,28] While mutations in the *SLC3A1* gene are typically inherited recessively in human patients, rare dominant traits have been documented.[23]

More Australian Cattle Dogs need to be studied to further corroborate the association of the clinical and metabolic effects with the mutation and to determine the mutant allele prevalence in the Australian Cattle Dog breed. While announcements for screening in the breed magazine and on the breed's listserve did not lead to any other cystinuric Australian Cattle Dogs, the fact that the cystinuric Australian Cattle Dogs studied here were from very different parts of the United States, ranging from the Southeast to the far Northwest, indicates the defect may be more widespread in the United States. Therefore, it might be appropriate to screen any Australian Cattle Dog intended for breeding or those showing clinical signs involving the urinary tract. The established DNA test using a simple fragment length size differentiation caused by the 6 bp deletion now can be used to detect affected dogs (Fig. 3) in addition to checking urine for cystine crystals or increased COLA concentrations, which identifies any cystinuric Australian Cattle Dogs but does not clearly differentiate between cystinuric heterozygotes and homozygotes.

Surprisingly, the mixed breed dog (MIXB 1) that formed cystine calculi early in life was found to be also homozygous for the same 6 bp deletion in *SLC3A1* (c.1095_1100del). However, phenotypically, with small stature and somewhat curly white fur, this dog did not resemble an Australian Cattle Dog. Interestingly, this mixed breed dog had more than 3 breed contributions from different grandparents (no Australian Cattle Dog), but was also highly

homozygous for the 312 analyzed SNPs obtained by the genetic mixed breed test, suggesting close inbreeding. Currently it is unclear how a mix between a Miniature Poodle, Chihuahua, and Shih Tzu could be so homozygous and more like a purebred dog. Moreover, this MIXB 1 dog was also homozygous for other variations within the *SLC3A1* gene and identical to the homozygous cystinuric Australian Cattle Dogs (Table 3). These findings suggest that the *SLC3A1* haplotypes in MIXB 1 and Australian Cattle Dogs are identical and they share a common ancestry rather than being caused by two separate events leading to the same mutation.

Last but not least, we describe here the first putative mutation in the *SLC7A9* gene associated with autosomal dominant cystinuria in **Miniature Pinschers** from Europe. It is a single base missense mutation (c.964G>A) changing the small hydrophobic glycine residue to the larger and charged basic amino acid arginine (G322R) in the transmembrane domain 9 of the light subunit $b^{o,+}$AT (Fig. 1C), which is highly conserved in all mammals and other vertebrates, including, chicken, a Xenopus species, and zebrafish (Fig 4B). According to $SIFT^{p}$ analysis, which predicts the consequences of gene mutations on protein function based on known protein and evolutionary conservation and three-dimensional structure, this substitution is not tolerated to sustain normal function, while protein stability seems unaffected.

In the family of Miniature Pinschers studied here, there were several cystinuric heterozygous dogs, but interestingly no homozygous cystinuric dogs were found. The mutation segregated with increased urine cystine levels through the entire pedigree, tracing back to a common heterozygous dam. Therefore this missense mutation appears to cause an autosomal dominant form of cystinuria (Fig. 2). In this study, there were no matings between heterozygous dogs, so at this time, we have not been able to observe the phenotype of a dog homozygous for the mutation. While for dominantly inherited traits, homozygosity for the

disease-causing allele can be lethal, this seems unlikely in cystinuria, as homozygous individuals with *SLC7A9* mutations have been observed in humans.[23,25]

In cystinuric humans, mutations in transmembrane domains have been described in the *SLC7A9* gene with homozygous individuals having severe clinical signs, while heterozygotes are mildly cystinuric and less commonly develop cystine calculi and at a later age.[23,29,30] It is unclear why this mutation causes a dominant type of cystinuria in dogs, although we can put forward at least one plausible hypothesis: because this missense mutation does not affect the binding site to rBAT, it likely results in a malfunctional transporter channel, rather than inhibiting heterodimerization of the two subunits. Having a malfunctioning and normal functioning $b^{0,+}$AT protein anchored in the membrane, may result in defective transport. Furthermore, it has been suggested that the COLA transporter is a heterotetramer formed from two heterodimers, potentially explaining abnormal or diminished transport when mutant and normal dimers interact.[31] In contrast, null mutations would not interfere and thus result in a recessive trait with intermediate normal activity. As indicated, all Miniature Pinschers heterozygous for the missense mutation in the *SLC7A9* gene were cystinuric, and some repeatedly developed cystine calculi independent of gender and neuter status. The degree of COLA-excretion was less severe than with the *SLC3A1* mutations described above, but surprisingly seemed to cause a relatively higher elevation of cystine compared to ornithine, lysine and arginine (Table 2). This is the reverse what is observed in cystinuric Newfoundlands and dogs of other breeds studied here, where the sum of the dibasic amino acids always exceeds that of cystine alone. Further studies of this mutation in Miniature Pinschers are needed to define the molecular mechanism in this first dominant canine cystinuria-associated mutation altering the $b^{0,+}$AT protein.

The prevalence of cystinuria in the Miniature Pinscher breed is currently unknown, but it is not a breed that has been reported to have a higher incidence of cystine stones by any

urolith analysis laboratory.[11,26] All cystinuric Miniature Pinschers studied belonged to the same family, and we identified a cystinuric heterozygote as a common ancestor. This dam was 11 years old when tested and its parents or other informative relatives were not available; hence we do not know, if this mutation originated in this dog, any of its ancestors or even in the line of the common sire with an unknown genotype (Fig. 2). Either sequencing of the DNA fragment surrounding the mutation or real time PCR based assays can be used for the screening of this mutation. Based upon our current knowledge, all Miniature Pinschers related to this family in Europe should be screened by mutation-specific DNA test, particularly when related to any cystinuric dogs. And because it is a dominant trait, all dogs homozygous or heterozygous for the mutation should be excluded from breeding.

We had previously divided cystinuria in dogs into 2 types, with type I referring to an *SLC3A1* mutation and autosomal recessive inheritance and non-type I cystinuria in Mastiffs and related breeds, Scottish Deerhounds, and Irish Terriers[a] exhibiting a milder degree of cystinuria solely in mature non-neutered male dogs (PH, UG, unpublished data).[14] The metabolic and genetic studies reported here in 3 additional canine breeds clearly expand our understanding of the heterogeneity of cystinuria in dogs. Moreover, the molecular basis of cystinuria in many breeds has not been elucidated. For example, the fact that the average age of stone-formation in the breeds with known associated mutations is much lower than the reported mean age of dogs when cystine calculi are removed (4.8 years to 5.6 years),[7,32,33] and that several breeds have been shown to be free from protein-coding mutations in the *SLC3A1* and *SCL7A9* genes,[12,21] indicates that the basis for additional heterogeneity in canine cystinuria remains to be elucidated.

And while cystinuria is also complex in humans and several classification systems have been proposed, no sex linked or sex limited forms of the disease have been identified.[3,23] We therefore propose here a classification system that encompasses both discriminating

aspects of the phenotype (for example, gender affected, androgen dependence, and mode of inheritance) and the gene associated with the disease (Table 4). We designate type I cystinuria when the disease shows autosomal recessive inheritance, Type II when it is autosomal dominant, and Type III for sex-limited, androgen dependent inheritance (PH, UG, unpublished data). Additional types can be assigned if found. Specific mutations within each type should lead to phenotypes that are sufficiently similar that the same medical management and breeding advice applies to all cases within that type. Involvement of the *SLC3A1* gene is indicated by adding –A, and similarly addendum of –B indicated involvement of mutations in *SLC7A9*. If no letter is appended, the genetic basis is unknown. Again, if additional genes are shown to be associated with cystinuria, additional letters can be assigned. Alternatively, stating simply the mode of inheritance and mutant gene could be used: autosomal recessive and dominant *SLC3A1* or dominant *SLC7A9* cystinuria and androgen-dependent cystinuria, where the molecular genetic defect has not been determined. These recent discoveries will have major clinical impacts including selection of the best clinical management and genetic control of cystinuria in future generations.

**Table 1**

| Gene | Exon | Forward Primer (5'-3') | Reverse Primer (5'-3') | Amplicon Size (bp) | Annealing Temperature in ºC |
|---|---|---|---|---|---|
| ***SLC3A1*** | **1** | *ccccttttacctttgccctttc* | *tactaataagcagctcccggc* | 739 | 60.0 |
| | **2-3** | *attttaggcatattggtttta* | *cagttgttgggtggtatgg* | 577 | 56.1 |
| | **4** | cattaggccagcagatagcatttt | caacatcgagtatatcaagtaacc | 388 | 63.0 |
| | **5** | *tcagcgtggtttcattcctt* | *tcattggtgtctcgtgctaaaa* | 471 | 62.3 |
| | **6** | *aaaaccccgtgagcattgaaag* | *aactggctgtgaaggataaactac* | 349 | 65 |
| | **7** | *atctggctcaattatgcttccta* | *ggcctggctcactaaataatcact* | 459 | 62.3 |
| | **8** | *gaaatctctgctgcctctgt* | *catttttaagcccccaacca* | 627 | 62.3 |
| | **9** | *aataactaggctggataaaagagg* | *ttgtgggcaggaaaggaaaac* | 655 | (touchdown) |
| | **10** | *tggccaagatgtagcagagca* | *cacacccccatcagaacagc* | 1,092 | 62.3 |
| ***SLC7A9*** | **1** | *tggagctccggggccttcacatac* | *cggcgcaccccaaagtcgttctac* | 1,103 | 60.0 |
| | **2** | *tccgcggcgccagaacaaatg* | *cccccgggcccgagcagac* | 536 | 62.3 |
| | **3-6** | *gaagccccatcacccgtctgtcct* | *gcccgcccgtctccgtgtcc* | 1,612 | 60.0 |
| | **7-8** | *gggggaccatcacgggctttctct* | *cggggcacctcggctgggcattta* | 843 | 64.2 |
| | **9** | *ggagtcccgggagccaaacc* | *cgcccccacccccacacc* | 665 | 66.1 |
| | **10** | *acccccatgctctcccctgtgtag* | *gggatgccgtgacgtctaactgt* | 709 | 64.2 |
| | **11** | *cgccgcttaaaactgcaacttcaa* | *ggcgcaaactccaccctaaacgat* | 520 | 64.2 |
| | **12** | *aaaggggggaaaagggagggagagg* | *tgacgggagcaaaggcagag* | 687 | 62.3 |
| | **13** | *cgcggggcagggacggtatt* | *gcggtgggggaggcagtaaaaca* | 602 | 69.0 |

**Table 1** – Primers and annealing temperatures used for the amplification of the *SLC3A1* and *SLC7A9* exons from gDNA.

**Table 2**

| Dog # | Gender (n) | Initial Signs (years) | Amino Acid Quantitation in the Urine (µmol/g Creatinine)* | | | | | Genotype |
|---|---|---|---|---|---|---|---|---|
| | | | Cystine | Ornithine | Lysine | Arginine | COLA | |
| **Labrador Retriever** | | | | | | | | ***SLC3A1 c.350delG*** |
| **LABR 1-3** | M/MC *(1/2)* | **0.7** | *355*** | *289*** | *5,708*** | *7,213*** | **13,565**** | **2 / 2** |
| **LABR 4** | F *(1)* | **7** | *5* | *1,099* | *2,143* | *81* | **3,328** | **2 / 2** |
| **LABX 1-2** | MC *(2)* | **1.1** | *834* | *557* | *4,660* | *4,200* | **10,250** | **2 / 2** |
| **Australian Cattle Dog** | | | | | | | | ***SLC3A1 c.1095_1100del*** |
| **AUCD 1, 3-4** | M/MC *(1/2)* | **0.7** | 716 *± 474* | 723 *± 179* | 5,852 *± 3,151* | 5,376 *± 1,163* | **12,667 ± 4,068** | **2 / 2** |
| **AUCD 6** | FS *(1)* | **4** | 822 | 510 | 4,052 | 4,852 | **10,236** | **2 / 2** |
| **AUCD 2,5** | M/MC *(1/1)* | **5** | 1,253**** | 214**** | 2,339**** | 228**** | **4,034**** | **2 / 1** |
| **MIXB 1** | MC *(1)* | **2** | 98 | 2,756 | 3,912 | 4,211 | **10,977** | **2 / 2** |
| **Miniature Pinscher** | | | | | | | | ***SLC7A9 c.964G>A*** |
| **MINP 1-4** | M/MC *(3/1)* | **1.7** | 386 *± 234* | 149 *± 14* | 255 *± 118* | *96 ± 66* | **737 ± 272** | **2 / 1** |
| **MINP 5-8** | F *(4)* | **4** | *485 ± 305* | *67 ± 5* | *355 ± 385* | *61 ± 38* | **918 ± 719** | **2 / 1** |
| **Normal Upper Limit** | | | *≤ 178* | *≤ 100* | *≤ 200* | *≤ 100* | ***≤500*** | **1 / 1** |

**Table 2** – Comparison of clinicalpathologic, metabolic and genetic findings in cystinuric Labrador Retrievers, Labrador mixed breed dogs, Australian Cattle Dogs and Miniature Pinschers (M=male, MC=male castrated, F=female, FS=female spayed, 1/1=normal genotype, 1/2=heterozygous for the mutation, 2/2=homozygous for the mutation);
*Mean value (± SD for ≥ 3 dogs); **COLA values only available for LABR 1 / AUCD 2

**Table 3**

| Gene | Exon/ Intron | SNP | Protein | Heterozygous | Homozygous |
|---|---|---|---|---|---|
| ***SLC3A1*** | **Exon 1** | ***c.350delG*** | **p.Gly117Alafs*41** | | **LABR 2**<br>**LABX 1** |
| | **Exon 1** | *c.378G>A* | Silent | | LABR 2<br>LABX 1 |
| | **Exon 6** | ***c.1095_1100del*** | **p.Thr366_Thr367del** | **AUCD 5** | **AUCD 1**<br>**MIXB 1** |
| | **Exon 7** | *c.1308G>C* | Silent | AUCD 5 | AUCD 1<br>MIXB 1<br>MINP 8 |
| ***SLC7A9*** | **Intron2** | *c.96+8T>C* | | LABR 2<br>LABX 1 | |
| | **Exon 4** | *c.267C>T* | Silent | AUCD 5 | AUCD 1<br>LABX 1 |
| | **Intron 4** | *c.561+11A>G*<br>*a. +13G>A* | | MIXB 1<br>MINP 8 | |
| | **Intron 7** | *c.759-8G>A* | | | LABX 1 |
| | **Exon 9** | c.928C>T | Silent | AUCD 1 | |
| | **Exon 9** | ***c.964G>A*** | **p.Gly322Arg** | **MINP 8** | |
| | **Intron 12** | *c.1409-25G>T* | | LABR 2 | LABX 1<br>AUCD 1 |

**Table 3** – Differences in the coding and flanking intronic sequences of *SLC3A1* and *SLC7A9* of 5 cystine stone formers (LABR 2, LABX 1, AUCD 1, AUCD 5, MIXB 1 and MINP 8) to the canine genome reference sequence.

**Table 4**

| **Phenotype** | | **Type I - A** | **Type II - A** | **Type II - B** | **Type III -** |
|---|---|---|---|---|---|
| **Inheritance** | | **Autosomal recessive** | **Autosomal dominant** | **Autosomal dominant** | **Sex limited** |
| **Gene** | | **SLC3A1** | **SLC3A1** | **SLC7A9** | **Undetermined** |
| **Gender** | | *Males and Females* | *Males and Females* | *Males and Females* | *Intact Adult Males* |
| **Androgen dependence** | | *No* | *No* | *No* | *Yes* |
| **COLA** *µmol/g creatinine* ***(normal ≤ 500)*** | **homozygous** | *≥ 8,000* | *≥ 8,000* | *nd* | *≤ 4,000* |
| | **heterozygous** | *≤ 500* | *≥ 3,000* | *≥ 700* | |
| **Breeds** | | *Newfoundland Landseer Labrador* | *Australian Cattle Dog* | *Miniature Pinscher* | *Mastiff & Related Breeds Scottish Deerhound Irish Terrier* |

**Table 4** – New expanded classification system for canine cystinuria according to the novel metabolic and genetic findings (nd = not determined).

## Fig. 1

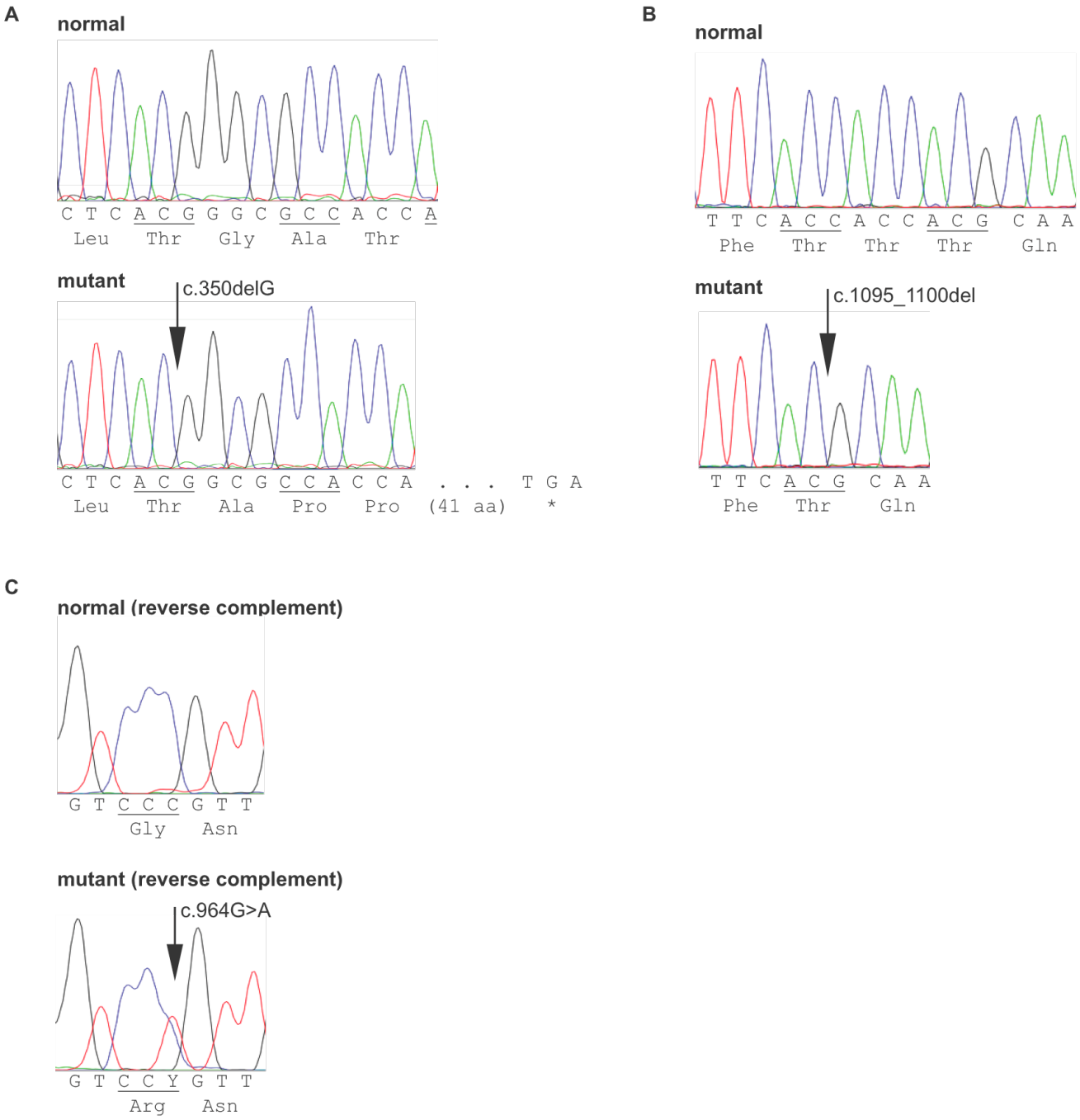


**Fig. 1** – Sequencing chromatograms displaying the mutations found and their effect on the protein sequence. **A)** A 1 bp deletion in *SLC3A1* exon 1 in purebred and mixed breed Labrador Retriever and causing a frameshift and a premature stop codon to truncate the translated protein. **B)** A 6 bp in frame deletion in *SLC3A1* exon 6 in Australian Cattle Dogs and a mixed breed dog deleting 2 threonines in a repeat of 3. **C)** A single nucleotide exchange in *SLC7A9* exon 9 found heterozygous in cystinuric Miniature Pinschers, changing the amino acid from glycin to arginine.

Fig. 2

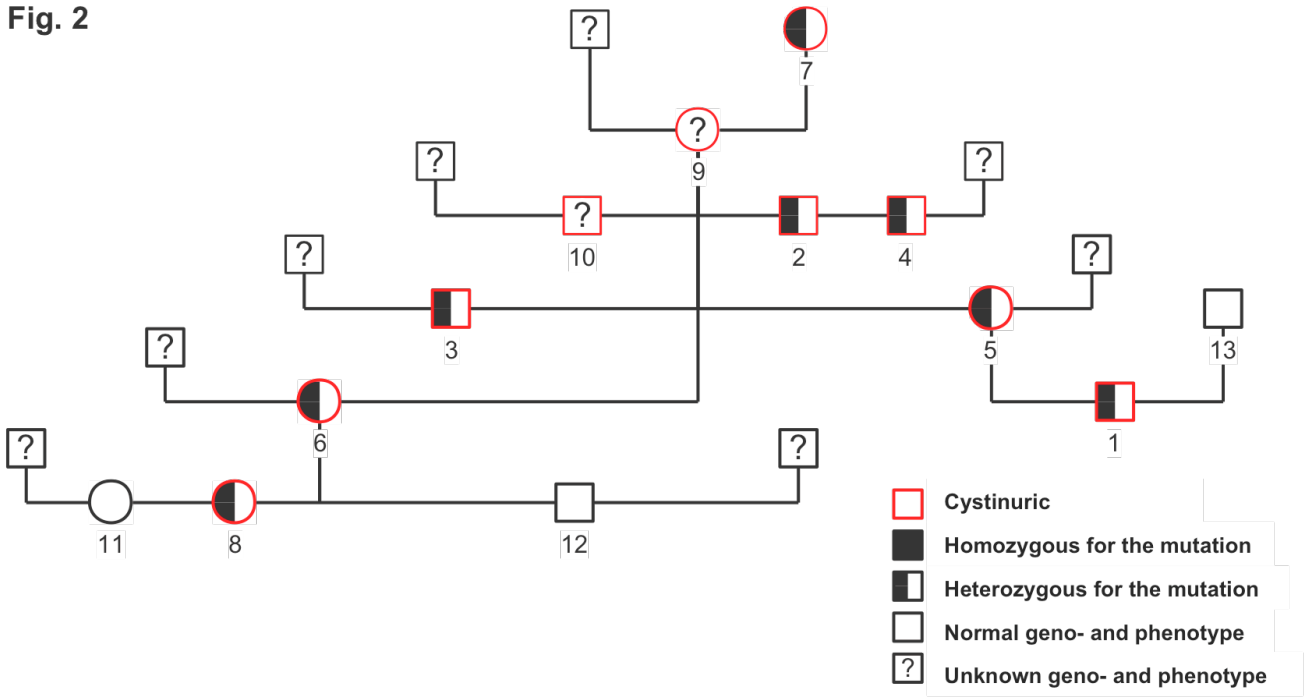


**Fig. 2** – Pedigree of the Miniature Pinscher family displaying the genotype and the phenotype of the dogs studied, showing their correlation and the inheritance in an autosomal dominant trait

Fig. 3

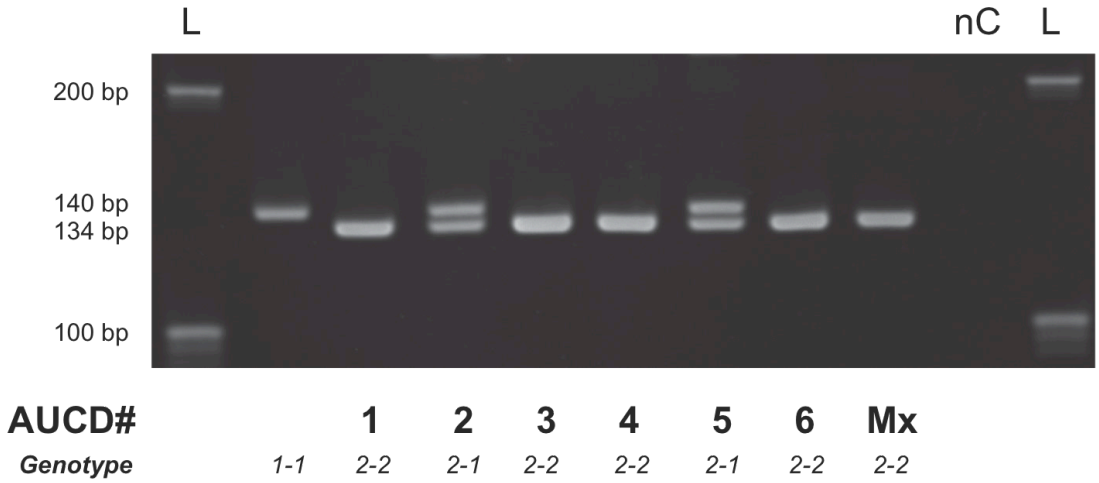


**Fig. 3** – Fragment length analysis on a 5% polyacrylamid gel, discriminating the normal DNA amplicon of 140 bp from the smaller fragment of 134 bp in presence of the 6 bp deletion in *SLC3A1* exon 6 found in cystinuric Australian Cattle Dogs (see AUCD #) and a mixed breed dog (here Mx)

**Fig. 4**

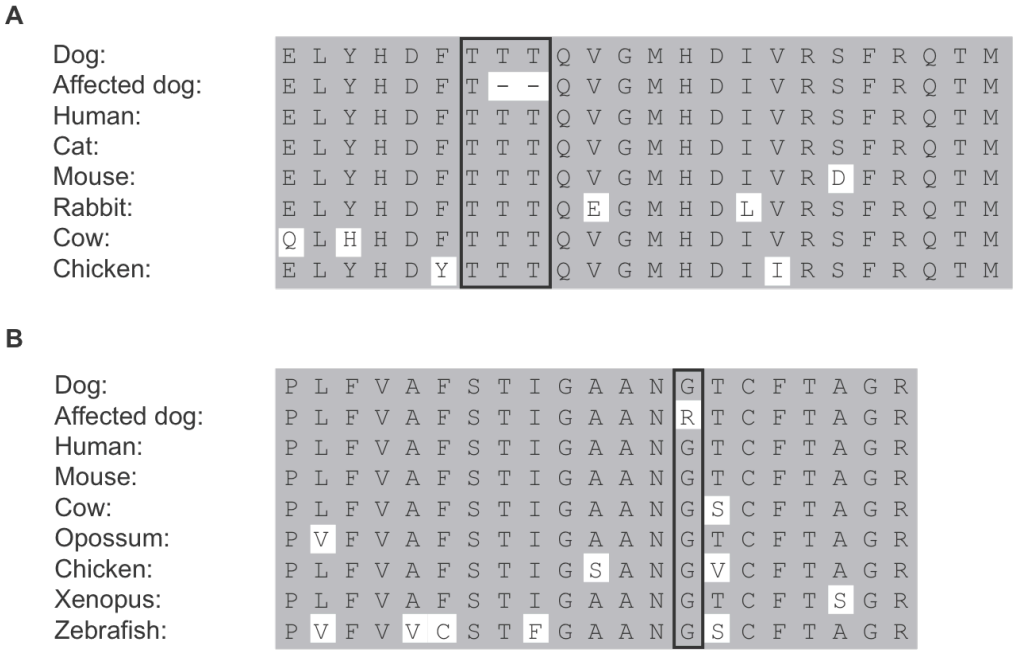


**Fig. 4** – Alignment of the amino acid sequences from different species, surrounding **A)** the deletion of two threonines, found in *SLC3A1* exon 6 in cystinuric Australian Cattle Dogs and a mixed breed dog, and **B**) the exchange of a glycine to an arginine, found in cystinuric Miniature Pinschers, both showing a highly conserved sequence in the protein.

## Footnotes

[a] Urs Giger, Jessica W. Lee, Cait Fitzgerald et al, Characterization Of Non-Type I Cystinuria In Irish Terriers, J Vet Int Med, 2011 ACVIM Forum Abstracts, 2011:25:718

[b] PennGen Laboratories, Philadelphia, PA

[c] Biochrom 32, Biochrom Ltd., Cambridge, UK

[d] QIAGEN, Hilden, Germany

[e] http://www.genome.ucsc.edu

[f] DNASTAR, Madison, WI

[g] Takara, Otsu, Shiga, Japan

[h] Invitrogen, Carlsbad, CA

[i] Merck, Darmstadt, Germany

[j] Eppendorf, Hamburg, Germany

[k] Applied Biosystems, Foster City, CA

[l] www.ncbi.nlm.nih.gov

[m] http://technelysium.com.au

[n] Mars Synbiotics, Gaithersburg, PA

[o] Captimer®, Biokanol Pharma, Rastatt, Germany

[p] http://sift.jcvi.org

# References


1. OMIA - Online Mendelian Inheritance in Animals. http://omia.angis.org.au/ [Last accessed: March 23, 2013].

2. Garrod A. The Croonian Lectures ON INBORN ERRORS OF METABOLISM. Lancet 1908;172:1-7.

3. Chillaron J, Font-Llitjos M, Fort J, et al. Pathophysiology and treatment of cystinuria. Nat Rev Nephrol 2010;6:424-434.

4. Palacin M, Goodyer P, Nunes V, et al. Cystinuria. In: Scriver CR, ed. The metabolic and molecular bases of inherited disease, 8th ed. New York: McGraw-Hill; 2001:4909-4932.

5. Calonge MJ, Gasparini P, Chillaron J, et al. Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. Nat Genet 1994;6:420-425.

6. Treacher RJ. Urolithiasis in the dog. II. Biochemical aspects. J Small Anim Pract 1966;7:537-547.

7. Hoppe A, Denneberg T. Cystinuria in the dog: clinical studies during 14 years of medical treatment. J Vet Intern Med 2001;15:361-367.

8. Osborne CA, Sanderson SL, Lulich JP, et al. Canine cystine urolithiasis. Cause, detection, treatment, and prevention. Vet Clin North Am Small Anim Pract 1999;29:193-211, xiii.

9. Lassaigne J. Observation sur l'existence de l'oxide cystique dans un calcul vesical du chien, et essai analytique sur la composition élémentaire de cette substance particulière. Ann Chim Phys 1823;23:328-334.

10. Ling GV, Franti CE, Ruby AL, et al. Urolithiasis in dogs. II: Breed prevalence, and interrelations of breed, sex, age, and mineral composition. Am J Vet Res 1998;59:630-642.

11. Osborne CA, Lulich JP, Kruger JM, et al. Analysis of 451,891 canine uroliths, feline uroliths, and feline urethral plugs from 1981 to 2007: perspectives from the Minnesota Urolith Center. Vet Clin North Am Small Anim Pract 2009;39:183-197.

12. Henthorn PS, Giger U. Cystinuria. In: Ostrander EA, Giger U, Lindblad-Toh K, eds. Cold Spring Harbor monograph series: The dog and its genome. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press; 2006:349-364.

13. Casal ML, Giger U, Bovee KC, et al. Inheritance of cystinuria and renal defect in Newfoundlands. J Am Vet Med Assoc 1995;207:1585-1589.

14. Giger U, Sewell AC, Lui J, et al. Update on Fanconi Syndrome and Cystinuria in Dogs: Amino Acidurias. In: ACVIM Forum, Denver, CO 2011.

15. Palacin M, Estevez R, Bertran J, et al. Molecular biology of mammalian plasma membrane amino acid transporters. Physiol Rev 1998;78:969-1054.

16. Palacin M, Nunes V, Font-Llitjos M, et al. The genetics of heteromeric amino acid transporters. Physiology (Bethesda) 2005;20:112-124.

17. Fernandez E, Carrascal M, Rousaud F, et al. rBAT-b(0,+)AT heterodimer is the main apical reabsorption system for cystine in the kidney. Am J Physiol Renal Physiol 2002;283:F540-548.

18. Henthorn PS, Liu J, Gidalevich T, et al. Canine cystinuria: polymorphism in the canine SLC3A1 gene and identification of a nonsense mutation in cystinuric Newfoundland dogs. Hum Genet 2000;107:295-303.

19. Brand E, Harris MM, Biloon S. Cystinuria. The excretion of a cystine complex which decomposes in the urine with the liberation of free cystine. J Biol Chem 1930;86:315-331.

20. Guidelines and recommendations for mutation nomenclature. http://www.hgvs.org/mutnomen/ [Last accessed: March 23, 2013].

21. Harnevik L, Hoppe A, Soderkvist P. SLC7A9 cDNA cloning and mutational analysis of SLC3A1 and SLC7A9 in canine cystinuria. Mamm Genome 2006;17:769-776.

22. Bannasch D, Henthorn PS. Changing paradigms in diagnosis of inherited defects associated with urolithiasis. Vet Clin North Am Small Anim Pract 2009;39:111-125.

23. Eggermann T, Venghaus A, Zerres K. Cystinuria: an inborn cause of urolithiasis. Orphanet J Rare Dis 2012;7:19.

24. Osborne CA, O'Brien TD, Ghobrial HK, et al. Crystalluria. Observations, interpretations, and misinterpretations. Vet Clin North Am Small Anim Pract 1986;16:45-65.

25. Bisceglia L, Fischetti L, Bonis PD, et al. Large rearrangements detected by MLPA, point mutations, and survey of the frequency of mutations within the SLC3A1 and SLC7A9 genes in a cohort of 172 cystinuric Italian patients. Mol Genet Metab 2010;99:42-52.

26. Houston DM, Moore AE. Canine and feline urolithiasis: examination of over 50 000 urolith submissions to the Canadian veterinary urolith centre from 1998 to 2008. Can Vet J 2009;50:1263-1268.

27. Broer S, Wagner CA. Structure-function relationships of heterodimeric amino acid transporters. Cell Biochem Biophys 2002;36:155-168.

28. Dello Strologo L, Pras E, Pontesilli C, et al. Comparison between SLC3A1 and SLC7A9 cystinuria patients and carriers: a need for a new classification. J Am Soc Nephrol 2002;13:2547-2553.

29. Edvardsson VO, Goldfarb DS, Lieske JC, et al. Hereditary causes of kidney stones and chronic kidney disease. Pediatr Nephrol 2013.

30. Font-Llitjos M, Jimenez-Vidal M, Bisceglia L, et al. New insights into cystinuria: 40 new mutations, genotype-phenotype correlation, and digenic inheritance causing partial phenotype. J Med Genet 2005;42:58-68.

31. Fernandez E, Jimenez-Vidal M, Calvo M, et al. The structural and functional units of heteromeric amino acid transporters. The heavy subunit rBAT dictates oligomerization of the heteromeric amino acid transporters. J Biol Chem 2006;281:26552-26561.

32. Brown NO, Parks JL, Greene RW. Canine urolithiasis: retrospective analysis of 438 cases. J Am Vet Med Assoc 1977;170:414-418.

33. Ling GV, Franti CE, Ruby AL, et al. Urolithiasis in dogs I: mineral prevalence and interrelations of mineral composition, age, and sex. American Journal of Veterinary Research 1998;59:624-629.

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# Curriculum Vitae

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## Schul- und Hochschulausbildung

| | |
|---|---|
| 1996 – 2005 | Altes Gymnasium, städtisches Gymnasium Flensburg; Abiturnote 1,7 (sehr gut) |
| 10/2005 – 10.03.2011 | Stiftung Tierärztliche Hochschule Hannover, Studium der Veterinärmedizin; Gesamtnote der Tierärztlichen Prüfung: 2,4 (gut) |
| 08/2012 – dato | Universität Zürich, Veterinärmedizinische Fakultät; Doktorat<br>Anfertigung der Dissertation am Department für Kleintiermedizin in Zusammenarbeit mit den PennGen Laboratories, Philadelphia, PA, USA, unter Leitung von Prof. Dr. Urs Giger |

## Tätigkeiten nach dem Studium:

| | |
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| 05/2011 – 07/2012 | Tierärztliches Zentrum für Diagnostik, Dres. Klein, Wölk und Müller, Flensburg, Deutschland;<br>Assistenztierärztin |
| 08/2012 – 04/2013 | University of Pennsylvania, Philadelphia, PA, USA,<br>School of Veterinary Medicine,<br>Section of Medical Genetics,<br>Research Fellow |
| 05/2013 - dato | Tierärztliches Zentrum für Diagnostik, Dres. Klein, Wölk und Müller, Flensburg, Deutschland;<br>Assistenztierärztin |